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Manual

HS-PCR Kit 5

Complete kit for hot-start PCR including *Taq* DNA polymerase and reaction buffers. Concentration 5 U/µl.

catalog #	size	concentration
1205-200H	200 U	5 U/µl
1205-1000H	1000 U	5 U/µl

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Advantages

- Complete kit recommended for standard hot-start PCR reaction.
- This kit contains the most popular used thermostable *Taq* DNA polymerase for hot-start PCR.

Description

***Taq* DNA polymerase** is thermophilic DNA polymerase purified from *E.coli* stream carrying a plasmid with a cloned gene encoding a DNA polymerase from *Thermus aquaticus*.
Enzyme catalysis incorporation of deoxynucleotides to 3' end of dsDNA at temp. 70-80 °C and presence of Mg^{2+} ions. *Taq* DNA polymerase lacks 3'-5' exonuclease activity (proofreading), but possesses weak 5'-3' exonuclease activity. Polymerase is blocked with anti-*Taq* monoclonal antibody. Full activation time requires 3-5 min of incubation at 95 °C.

KU buffer increases the specificity of the PCR reaction for DNA templates with secondary structures and GC pairs. Using KU buffer it's necessary to prepare a control reaction without KU buffer.

"I" reaction buffer contains Mg^{2+} ions at a concentration ensuring satisfactory results in most experimental systems. Optimization of the concentration of Mg^{2+} ions in the reaction is the possibility of using an "III" reaction buffer (without Mg^{2+} ions) and adding an appropriate amount of Mg^{2+} ions to it in the form of $MgCl_2$ included in the kit.

Contents

	1205-200H	1205-1000H	storage
RUN-HS polymerase	200 U	1000 U	-20 °C
dNTP Mix (10 mM)	200 µl	4 x 200 µl	-20 °C
10x "I" buffer (with Mg^{2+} ions) 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl, pH 8.5, 15 mM $MgSO_4$, 1% Triton X-100	1.5 ml	4 x 1.5 ml	-20 °C
10x "III" buffer (without Mg^{2+} ions) 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl, pH 8.5, 1% Triton X-100	1.5 ml	4 x 1.5 ml	-20 °C
5x KU buffer no DMSO, no toxic reagents	2 ml	4 x 2 ml	-20 °C
6x loading buffer	1 ml	1 ml	-20 °C
$MgCl_2$ (25 mM)	1.5 ml	2 x 1.5 ml	-20 °C
ultrapure water	5 ml	4 x 5 ml	-20 °C

Example PCR protocol

1. Thaw all components on ice, gently mix by inverting the tubes and briefly centrifuge. Place the tube on ice.
2. Place PCR tube on ice and add:

	PCR reaction volume
component	**25 µl**
10x "I" buffer or "III" buffer	2.5 µl
dNTP Mix (10 mM)	200-250 µM (0.5-0.6 µl)
primer 1	0.1-0.5 µM
primer 2	0.1-0.5 µM
RUN-HS polymerase	1-5 U
DNA template	10 pg -1 µg
5x KU buffer (option)	2.5-5 µl
$MgCl_2$ (option)	depending on the needs
6x loading buffer (option)	depending on the needs
ultrapure water	up to 25 µl

3. Gently mix the sample and briefly centrifuge.
4. Place the tube in the thermocycler and start the PCR programme.

   An example amplification profile for products up to 1000 bp:

step	temperature	time
initial denaturation	95 °C	3-5 min
25-45 cycles	95 °C 50-68 °C 72 °C	15 s 30-60 s 1 min
final incubation	72 °C	10 min

5. PCR product store in a refrigerator or freezer until later use.

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